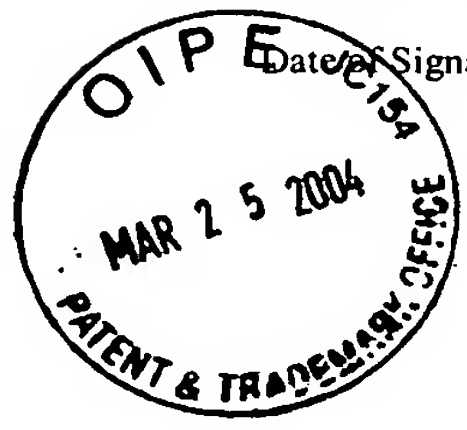


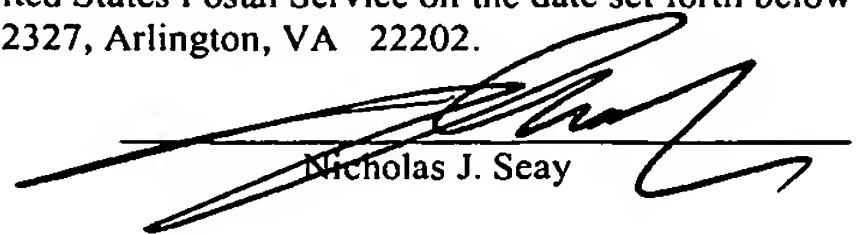
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Date of Signature and Deposit: March 22, 2004

  
Nicholas J. Seay

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Frederick R. Blattner  
Gyorgy Posfai  
Christopher D. Herring  
Guy Plunkett III

Date: March 22, 2004

Serial No.: 10/057,582

Group Art Unit:

Filed: 01/23/2002

Examiner:

Title: BACTERIA WITH REDUCED GENOME

File No.: 960296.95726

**INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97 and 1.98**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

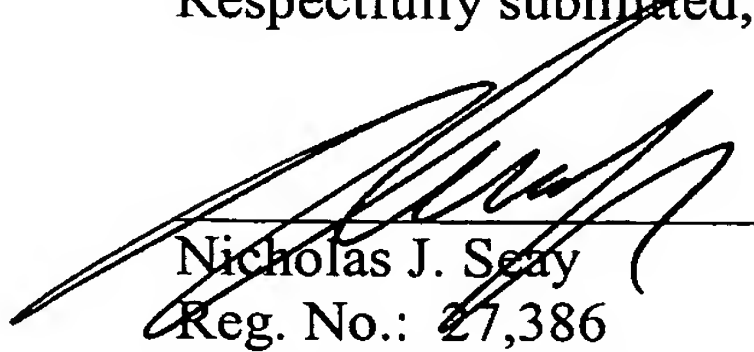
In compliance with 37 C.F.R. § 1.97 and the continuing duty of disclosure under 37 C.F.R. § 1.56, the applicants call to the attention of the examiner references listed on the attached Forms PTO/SB/08A and PTO/SB/08B. The applicants submit copies for all the references, except for references C12, C39 and C42 which are not readily available to us. The applicants also do not submit copies of references C7 and C38 because these references are lab manuals which simply describe standard procedures of molecular biology/biochemistry well known to a person of ordinary skill in the relevant art.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes art under 35 U.S.C. §102 or §103.

The Information Disclosure Statement is submitted before a first Office action on the merits for the above-identified patent application. Consequently, pursuant to 37 C.F.R. § 1.97 (b) (4), we do not believe any fee is due with the submission of this Information Disclosure Statement. However, should any fees be deemed necessary in connection with the

filing of this document, the Commissioner is hereby authorized to deduct any such fees from our Deposit Account No. 17-0055.

Respectfully submitted,



Nicholas J. Seay

Reg. No.: 27,386

Attorney for Applicants

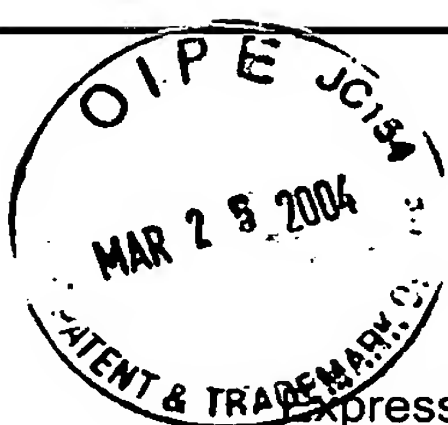
QUARLES & BRADY LLP

P.O. Box 2113

Madison, WI 53701

TEL 608/251-5000

FAX 608/251-9166



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# FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ ) 0.00

**Complete if Known**

Application Number	10/057,582
Filing Date	01/23/2002
First Named Inventor	Frederick R. Blattner
Examiner Name	
Art Unit	1636
Attorney Docket No.	960296.95726

**METHOD OF PAYMENT** (check all that apply)☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None☒ Deposit Account:Deposit Account Number  
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☒ Charge fee(s) indicated below ☒ Credit any overpayments☒ Charge any additional fee(s) or any underpayment of fee(s)☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.**FEE CALCULATION****1. BASIC FILING FEE**

Large Entity		Small Entity		Fee Description	Fee Paid
Fee Code	Fee (\$)	Fee Code	Fee (\$)		
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	
SUBTOTAL (1)					(\$ ) 0.00

**2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE**

		Extra Claims		Fee from below		Fee Paid
Total Claims	<input type="text"/>	-20*	= <input type="text"/>	X	<input type="text"/>	= 0.00
Independent Claims	<input type="text"/>	-3**	= <input type="text"/>	X	<input type="text"/>	= 0.00
Multiple Dependent					<input type="text"/>	= <input type="text"/>

Large Entity		Small Entity		Fee Description
Fee Code	Fee (\$)	Fee Code	Fee (\$)	
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$ ) 0.00

\*\*or number previously paid, if greater; For Reissues, see above

**FEE CALCULATION** (continued)**3. ADDITIONAL FEES**

Large Entity Small Entity

Fee Code	Fee (\$)	Fee Code	Fee (\$)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) \_\_\_\_\_

\*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$ ) 0.00

**SUBMITTED BY**

(Complete (if applicable))

Name (Print/Type)

Nicholas J. Seay

Registration No.  
(Attorney/Agent)

27,386

Telephone

608/251-5000

Signature

Date

March 22, 2003

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This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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PTO/SB/08A (10-01)

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				<b>Application Number</b>	10/057,582
				<b>Filing Date</b>	January 23, 2002
				<b>First Named Inventor</b>	Frederick Blattner
				<b>Group Art Unit</b>	1636
				<b>Examiner Name</b>	To be assigned
<b>Sheet</b>	1	<b>of</b>	6	<b>Attorney Docket Number</b>	960296.95726

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. <sup>1</sup>	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number – Kind Code <sup>2</sup> (if known)			
	A1	US-5,747,662	05-05-1998	Simmons et al.	
	A2	US-5,578,464	11-26-1996	Lunn et al.	
	A3	US-5,824,502	10-20-1998	Honjo et al.	
	A4	US-5,962,327	10-05-1999	Dujon et al.	
	A5	US-6,015,709	01-28-2000	Natesan	
	A6	US-6,022,952	02-08-2000	Weiner et al.	
	A7	US-6,117,680	09-12-2000	Natesan et al.	
	A8	US-6,238,924	05-29-2001	Dujon et al.	
	A9	US-6,335,178	01-01-2002	Weiner et al.	
	A10	US-6,372,476	04-16-2002	Belguith et al.	
	A11	US-6,410,273	06-25-2002	Crouzet et al.	
	A12	US-6,509,156	01-21-2003	Stewart et al.	

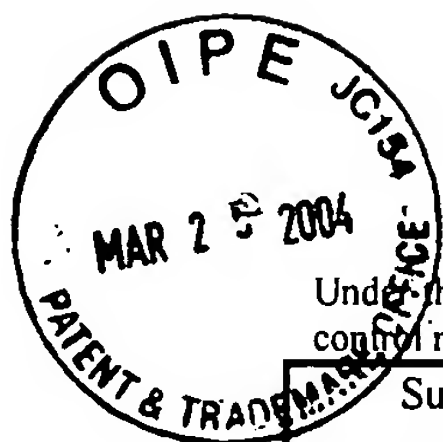
FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. <sup>1</sup>	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T <sup>6</sup>
		Country Code <sup>3</sup> – Number <sup>4</sup> – Kind Code <sup>5</sup> (if known)				
	B1	WO 96/14,408	05-17-1996	Chouluka		
	B2	WO 02/14,495 A2	02-21-2002	Court et al.		
	B3	EP 0177343	04-09-1986	Lawrence et al.		

<b>Examiner Signature</b>		<b>Date Considered</b>	
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<sup>1</sup> Applicant's unique citation designation number (optional). <sup>2</sup> See Kinds codes of USPTO Patent Documents as [www.uspto.gov](http://www.uspto.gov) or MPEP 901.04. <sup>3</sup> Enter Office that issued the document, by the two-letter code (WIPO Standard ST. 3). <sup>4</sup> For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. <sup>5</sup> Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. <sup>6</sup> Applicant is to place a check mark here if English language Translation is attached.

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				<b>Application Number</b>	10/057,582
				<b>Filing Date</b>	January 23, 2002
				<b>First Named Inventor</b>	Frederick Blattner
				<b>Group Art Unit</b>	1636
				<b>Examiner Name</b>	To Be Assigned
<b>Sheet</b>	2	<b>of</b>	6	<b>Attorney Docket Number</b>	960296.75726

<b>OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS</b>			
Examiner Initials*	Cite No. <sup>1</sup>	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher city and/or country where published	T <sup>2</sup>
	C1	Balbas (2001). <b>Understanding the art of producing protein and non-protein molecules in E. coli.</b> Molec Biotechnol 19: 251-267	
	C2	Baneyx (1999). <b>Recombinant protein expression in E. coli.</b> Current Opinion in Biotech 10: 411-421	
	C3	Berry et al. (2002). <b>Application of metabolic engineering to improve both production and use of biotech indigo.</b> J Indust Micro & Biotech 22: 127-133	
	C4	Blattner et al. (1997). <b>The complete genome sequence of Escherichia coli K-12.</b> Science 277:1453-74	
	C5	Blaudeck et al. (2001). <b>Specificity of single peptide recognition in TAT-dependent bacterial protein translocation.</b> J. Bacteriology 183:604-610	
	C6	Court et al. (2002). <b>Genetic engineering using homologous recombination.</b> Annu. Rev. Genet. 36: 361-88	
	C7	Current Protocols in Molecular Biology (1994). 16.6.1-16.6.14 (Copyrighted 2000 by John Wiley et al. and Sons)	
	C8	Danese et al. (1998). <b>Targeting and assembly of periplasmic and outer-membrane proteins in Escherichia coli.</b> Annu. Rev. Genet. 32:59-94	
	C9	Datsenko et al (2000). <b>One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products.</b> Proc. Natl. Acad. Sci. 97:6640-6649	
	C10	Degryse (1995). <b>Evaluation of Escherichia coli <i>recBC sbcBC</i> mutants for cloning by recombination in vivo.</b> J. Biotechnology 39: 181-187	

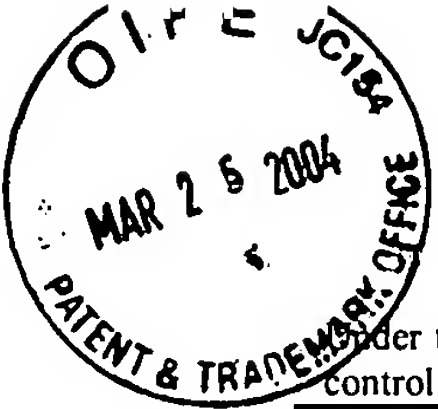
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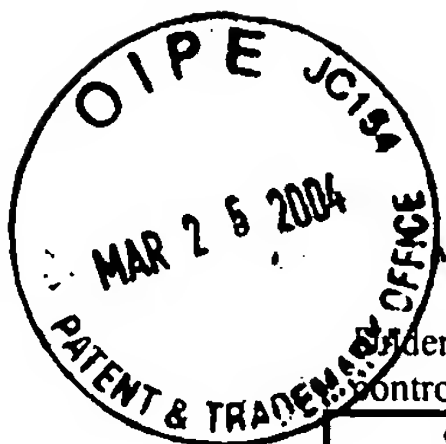
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				January 23, 2002	
				<b>First Named Inventor</b>	
Frederick Blattner					
<b>Group Art Unit</b>					
1636					
<b>Examiner Name</b>					
To Be Assigned					
<b>Attorney Docket Number</b>					
960296.75726					

Sheet	3	of	6
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C11	DeLisa et al. (2001). <b>Quorum sensing via AI-2 communicates the metabolic burden associated with heterologous protein production in E. coli.</b> Biotech Bioeng 75(4): 439-450
C12	Feher et al. (September 11, 2002). <b>Engineering a reduced Escherichia coli genome.</b> Conference Abstract.
C13	Fekkes et al. (1999). <b>Protein targeting to the bacterial cytoplasmic membrane.</b> Microbiol. Mol. Biol. Rev. 63:161-193
C14	Gill et al. (2000). <b>A comparative study of global stress gene regulation in response to overexpression of recombinant proteins in E.coli.</b> Metabolic Engineering 2: 178-189.
C15	Hanahan et al. (1983). <b>Studies on transformation of Escherichia coli with plasmids.</b> J. Mol. Biol. 166(4):557-580
C16	Hannig (1998). <b>Strategies for optimizing heterologous protein expression in Escherichia coli.</b> Trends Biotechnol. 16(2):54-60
C17	Hayashi et al (2001). <b>Construction of a genetic linkage map of the model legume Lotus japonicus using an intraspecific F2 population.</b> DNA Research 8: 11-22
C18	Hockney (1994). <b>Recent developments in heterologous protein production in Escherichia coli.</b> Trends Biotechnol. 12(11):456-632
C19	Hynds et al. (1998). <b>The sec-independent twin-arginine translocation system can transport both tightly folded and malfolded proteins across the thylakoid membrane.</b> J. Biol. Chem. 273:34868-34874
C20	Kitamura (1995). <b>DNA sequence changes in mutations in the ton B gene on the chromosome of Escherichia coli K-12: insertion elements dominate the spontaneous spectra.</b> Jpn J Genet 70: 35-46
C21	Kolisnychenko et al. (2002). <b>Engineering a reduced Escherichia coli genome.</b> Genome Research 12:640-647
C22	Koob et al.. <b>Minimizing the genome of Escherichia coli.</b> Ann. N.Y. Acad. Science

<b>Examiner Signature</b>		<b>Date Considered</b>	
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PTO/SB/08B (10-01)

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				<b>Application Number</b>	10/057,582
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				<b>First Named Inventor</b>	Frederick Blattner
				<b>Group Art Unit</b>	1636
				<b>Examiner Name</b>	To Be Assigned
<b>Sheet</b>	4	<b>of</b>	6	<b>Attorney Docket Number</b>	960296.75726

	C23	Koonin (2000). <b>How many genes can make a cell: The minimal-gene-set concept.</b> Ann Rev Genom Hum Genet 1: 99-116	
	C24	Lee (1996). <b>High cell-density culture of Escherichia coli.</b> TIBTECH 14:98-103	
	C25	Murphy (1998). <b>Use of bacteriophage <math>\lambda</math> recombination functions to promote gene replacement in <i>Escherichia coli</i>.</b> J. Bacteriol. 180: 2063-2071	
	C26	Muyrers et al. (1999). <b>Rapid modification of bacterial artificial chromosomes by ET-recombination.</b> Nucl. Acids. Res. 27: 1555-1557	
	C27	Neidhardt et al. (1974). <b>Culture medium for Enterobacteria.</b> J. Bacteriol. 119:736-747	
	C28	Oliner et al. (1993). <b>In vivo cloning of PCR products in E. coli.</b> Nucleic Acids Res. 2(22): 5192-7	
	C29	Otto et al. (2002). <b>Surface sensing and adhesion of E.coli controlled by the Cpx-signaling pathway.</b> Proc. Nat. Acad. Sci. US 99(4): 2287 2292	
	C30	Perna et al. (2001). <b>Genome sequence of enterohaemorrhagic Escherichia coli O157:H7.</b> Nature 409:529-533	
	C31	Perna et al. (2002). <b>The genomes of Escherichia coli K-12 and pathogenic E. coli.</b> Pathogenic E.coli Paradigm for Bacterial pathogenesis, M.S. Donnenberg, Editor. Academic Press	
	C32	Pfeifer et al. (2001). <b>Biosynthesis of complex polyketides in a metabolically engineered strain of E. coli.</b> 291: 1790-1792	
	C33	Posfai et al. (1997). <b>Versatile insertion plasmids for targeted genome manipulations in bacteria: isolation, deletion, and rescue of the pathogenicity island LEE of the Escherichia coli O157:H7 genome.</b> J. Bacteriol. 179: 4426 -4428	
	C34	Posfai et al. (1999). <b>Markerless gene replacement in Escherichia coli stimulated by a double-strand break in the chromosome.</b> Nucl. Acids Res. 27:4409-4415	

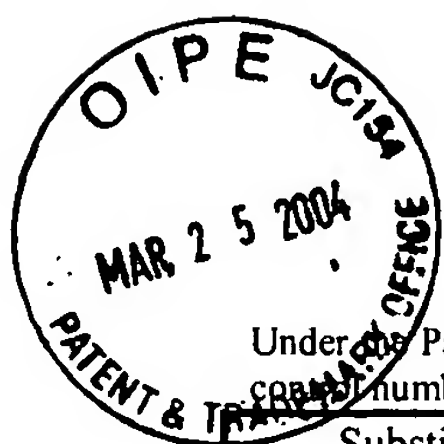
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<b>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</b>  (use as many sheets as necessary)				<b>Application Number</b>	10/057,582
				<b>Filing Date</b>	January 23, 2002
				<b>First Named Inventor</b>	Frederick Blattner
				<b>Group Art Unit</b>	1636
				<b>Examiner Name</b>	To Be Assigned
<b>Sheet</b>	5	<b>of</b>	6	<b>Attorney Docket Number</b>	960296.75726

	C35	Pugsley (1993). <b>The complete general secretory pathway in gram-negative bacteria.</b> Microbiol. Rev. 57:50-108	
	C36	Reisenberg (1991). <b>High cell density cultivation of E.coli at controlled specific growth rate.</b> J. Biotech 20(1): 17-27	
	C37	Ritz et al. (2001). <b>Roles of thiol redox pathways in bacteria.</b> Annu Rev Microbiol 55: 21-48	
	C38	Sambrook et al.(1989). <b>Molecular Cloning: a Laboratory Press,</b> Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.	
	C39	Sanford (16-17 May 2002). <b>The role of Biotechnology in Industrial Sustainability in section "Factory of tomorrow."</b> Conference, Antwerp	
	C40	Santini et al. (1998). <b>A novel sec-independent periplasmic protein translocation pathway in Escherichia coli.</b> EMBO J. 17:101-112	
	C41	Sargent et al. (1998). <b>Overlapping functions of components of a bacterial Sec-independent protein export pathway.</b> EMBO J. 17:3640-50	
	C42	Schaechter et al. (1997). <b>Introduction.</b> In Escherichia coli and Salmonella (ed. Neidhart, FC et al.) 1-2. ASM Press, Washington, DC.	
	C43	Selinger et al. (2000). <b>RNA expression analysis using a 30 base pair resolution Escherichia coli genome array.</b> Nat Biotechnol 18(12): 1262-1268	
	C44	Simmons et al. (1996). <b>Translational level is a critical factor for secretion of heterologous proteins in E. coli.</b> Nature 14: 629-634	
	C45	Sing-Gasson et al. (1999). <b>Maskless fabrication of light-directed oligonucleotide microarrays using a digital micromirror array.</b> Nat Biotechnol. 17(10): 974 978	
	C46	Swartz (2001). <b>Advances in E. coli production of therapeutic proteins.</b> Curr Opinion in Biotech 12: 195-201	

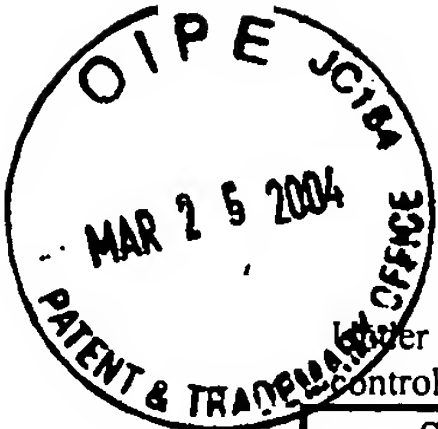
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<b>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</b>  (use as many sheets as necessary)				<b>Application Number</b>	10/057,582
				<b>Filing Date</b>	January 23, 2002
				<b>First Named Inventor</b>	Frederick Blattner
				<b>Group Art Unit</b>	1636
				<b>Examiner Name</b>	To Be Assigned
<b>Sheet</b>	6	<b>of</b>	6	<b>Attorney Docket Number</b>	960296.75726

	C47	Thomas et al. (2001). <b>Export of active green fluorescent protein to the periplasm by the twin-arginine translocase (Tat) pathway in <i>Escherichia coli</i>.</b> Mol. Micro. 39(1):47-53	
	C48	Venkatesan et al. (2001). <b>Complete DNA Sequence and analysis of the large virulence plasmid of <i>Shigella flexneri</i>.</b> Infection of Immunity 3271-3285	
	C49	Weiner et al. (1998). <b>A novel and ubiquitous system for membrane targeting and secretion of cofactor-containing proteins.</b> Cell 93:93-101	
	C50	Welch et al. (2002). <b>Extensive mosaic structure revealed by the complete genome sequence of uropathogenic <i>Escherichia coli</i>.</b> Proc. Natl. Acad. Sci. USA 99(26): 17020-17024	
	C51	Yu et al. (2000). <b>An efficient recombination system for chromosome engineering in <i>Escherichia coli</i>.</b> Proc. Natl. Acad. Sci. USA 97: 5978-5983	
	C52	Yu et al. (2002). <b>Minimization of the <i>Escherichia coli</i> genome using a Tn5-targeted Cre/LoxP excision system.</b> Nature Biotech. 20:1018-1023	
	C53	Zhang et al. (1998). <b>A new logic for DNA engineering using recombination in <i>Escherichia coli</i>.</b> Nature Genetics 20: 123-128	
	C54	Zhang et al. (2000). <b>DNA cloning by homologous recombination in <i>Escherichia coli</i>.</b> Nature Biotechnology 18: 1314-1317	
	C55	Zhang et al. (2003). <b>Phage annealing proteins promote oligonucleotide-directed mutagenesis in <i>Escherichia coli</i> and mouse ES cells.</b> BMC Molecular Biology 4: 1	
	C56	Yu et al. (Oct. 2002), <b>Minimization of the <i>Escherichia coli</i> genome using Tn5-targeted Cre/loxP excision system,</b> Nature Biotechnology, Vol. 20, pp. 1018-1023.	

<b>Examiner Signature</b>		<b>Date Considered</b>	
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